XLVIII. THE EFFECT OF ULTRA-VIOLET LIGHT ON THE CALCIUM AND PHOSPHORUS METABOLISM OF THE LACTATING ANIMAL.

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IN growth, in pregnancy and in lactation the animal requirements for calcium and phosphorus are relatively great. This is particularly the case in farm animals in many of which powers of rapid growth, frequent pregnancies and heavy lactations are the characteristics sought after. Apart from the composition of the diet, ultra-violet light would seem to be the factor which most profoundly affects the metabolism of calcium and phosphorus. In 1924 we [Orr, Magee and Henderson, 1924] showed that in the case of the growing pig irradiation by a carbon arc lamp increased the absorption of calcium and phosphorus as estimated by balance experiments and subsequent analysis of the bones. The work of Maynard, Goldberg and Miller [1925] on the prevention of "stiffness" in swine by exposure to sunlight largely confirms our results.

It is generally believed that in a milking animal there is a storing up of calcium and phosphorus during the pre-lactation stage. In heavy milkers they are secreted in such amount that during early lactation an animal fed on an ordinary practical ration is commonly on a negative balance, particularly with regard to calcium. The difficulty of reducing this negative balance has been frequently noted. Thus Forbes *et al.* [1922] found that, despite superabundant supplies of calcium, phosphorus and other minerals, the calcium balances remained negative in the first half of lactation. Hart, too, and his associates [1922, 2] showed that, whilst substituting alfalfa hay for timothy hay in the ration reduced the negative calcium balances of milking cows, it did not render them positive. A supplement of bone meal added to timothy hay had the same effect. On the other hand, Hart and his co-workers [1922, 1] had previously succeeded in obtaining positive calcium balances by feeding alfalfa hay. The non-success of the later experiments they attribute to the method of curing the hay and they believe that there had been a "destruction during the curing process of the vitamin assisting calcium assimilation." This would help to explain the earlier work of Steenbock and Hart [1913] in which the good effect of green pasture on the calcium retention of milking goats was noted. The view is further supported by an investigation by Gaessler and McCandlish [1923] in which positive calcium balances were

obtained on feeding fresh alfalfa but negative balances when old alfalfa was used. Further, Miller, Brandt and Jones [1924] have demonstrated the beneficial effect of green kale upon the mineral balances in early lactation. Munroe [1924] has obtained calcium storage in liberally milking cows, a result which he tentatively ascribes to "some organic factor assisting calcium assimilation" in the large amount of clover hay being fed. Hart, Steenbock and Hoppert [1921] have shown that in milking goats the administration of cod-liver oil (5 to 10 cc. per day) may convert negative to positive calcium balances. The oil, however, was not very well tolerated.

Arising out of metabolic experiments with lactating goats, Hunt, Winter, Schultz and Miller [1923] suggest that "the effect of green feed in causing the difference in assimilation of calcium over that of dry feed is due to the fact, other than the vitamin theory, that the calcium in the cells of green plants and hay not exposed to an excessive drying is more highly dispersed and therefore better digested and assimilated than the calcium in dry hay." They believe this theory to be strengthened by the fact that tricalcium phosphate precipitated in a starch paste and added to a grain and hay ration brought about positive balances in lactating goats [Hunt, Winter and Miller, 1923].

Reference must also be made to the work of Meigs and his associates [1919]. They are of opinion that the experimental procedure as carried out in metabolic experiments with cows is largely responsible for the negative calcium balances so frequently found.

In brief, then, it has been shown or suggested that the following factors influence the absorption of calcium and phosphorus by the lactating animal: (1) stage of lactation; (2) calcium and phosphorus content of the ration; (3) nature of the organic constituents of the diet, either as regards their content of anti-rachitic factor, or as regards the digestibility of their calcium and phosphorus; (4) a nervous factor.

In none of the above-described experiments had the effect of sunlight or its absence been directly considered. It seemed to us that this might be a factor further complicating an already complicated problem. In view of our previous findings as regards the stimulus to calcium and phosphorus retention induced by irradiation of the growing pig, several experiments on the effect of ultra-violet light on the mineral metabolism of lactating goats were carried out. Meanwhile Hart, Steenbock and Elvehjem [1924] published results which showed that ultra-violet irradiation may convert negative balances of calcium and phosphorus in the lactating goat to positive balances. In a preliminary note we [Orr, Magee and Henderson, 1925] described three of our experiments which were then completed. The present paper describes these investigations in somewhat greater detail and also a fourth experiment which was in progress when the note was published.

EXPERIMENTAL WORK.

The technique employed throughout the four experiments was in general the same. Any minor variations are noted under the respective experiments.

Method.

Animals. Two lactating goats, of a Toggenberg strain not pure bred, were used, one a black and white animal, the other light grey in colour. Their coats were not long and as stated were comparatively light in colour but in no cases were the animals closely cropped or shaved.

Housing. Prior to each experiment the animal concerned occupied an inside pen for some time. Thereafter it was housed in a metabolism cage in the animal room. All light entering the room traversed glass. Before analyses were begun several days were allowed for the animal to become accustomed to its environment and diet.

Diet. This varied as shown under each experiment, but in the main consisted of a ration of hay and concentrates. Distilled water was given *ad lib.* Before each experiment the calcium, phosphorus and nitrogen content of each constituent of the diet was determined. In the case of the hay, the moisture content was also estimated. On the day the samples were taken for analysis the daily rations of hay for the whole duration of the experiment were weighed out and stored in separate bags; thus difficulties due to variations in moisture content from day to day were avoided. Any hay scraps left by the goat during a collection period were weighed and the percentage moisture estimated. From this the weight of calcium, phosphorus and nitrogen unconsumed was calculated and allowance made.

Collection of excreta and milk. The general routine employed in the collection of excreta was that in common use at the Rowett Institute [Husband, Godden and Richards, 1923]. Urine and faeces were measured and weighed daily and one-quarter of the urine and one-tenth of the faeces kept in a cool place, using a few drops of toluene as preservative. These aliquot portions were thoroughly mixed at the end of the 4 days, which generally constituted a collection period, and samples were taken for analysis.

The goat was milked twice daily, at 9 a.m. and 5 p.m. The same milker continued throughout an experiment. The volume at each milking was recorded and the milk with the addition of a few drops of formalin stored in a cool place till 4 days' milkings had been collected. The analyses were carried out on samples from this 4 days' total.

Analyses. Calcium was precipitated as oxalate and estimated volumetrically. For phosphorus the modified Pemberton-Neumann method was employed [Richards and Godden, 1924] and in Exp. 4 Briggs' modification of the Bell-Doisy technique was also used.

Irradiation. In all four experiments an irradiation period (varying from 14 to 21 days) was interposed between a pre- and a post-period of about equal duration.

The irradiation period consisted of 4 hours' daily exposure to the carbon arc at 4 feet distance. The current consumption at 230 volts a.c. was 10 amperes when the lamp was consuming 45¹.

Experiment 1.

The light grey goat, in the late lactation stage and yielding about 120 cc. per day, was used. The periods were as follows: (1) a preliminary period of 28 days, during which the goat was in an inside pen; (2) a pre-period of 11 days; (3) an irradiation period of 14 days and (4) a short post-period of 5 days. Collection and analyses were carried out on the 3rd and 4th days alternately, except in the post-period when they were on the 3rd and 5th days of that period.

Diet.

Food	Weight in g.	% CaO	% P ₂ O ₅
Hay	900	1.2810	0.4019
Oats	70	0.1158	0.6679
Linseed meal	25	0.3965	0.9306
Maize meal	150	0.0110	0.5395
Total g. per day	1145	11.7257	5.1263

Results. It will be seen from Table II, Exp. 1, and also from the accompanying graph that whilst the CaO and P₂O₅ balances remained negative throughout the experiment the CaO balance was less negative during the irradiation period. The reverse is true of the P₂O₅ balance. No significant change is seen in the composition of the urine or in the volume and composition of the milk during irradiation. From the following figures, however, and from Table I it will be seen that such increase as took place in the CaO retention during irradiation is largely due to a decreased excretion in the faeces. Thus the average daily excretion of CaO in the faeces was 11.77, 11.53 and 11.69 g. in the pre-period, the irradiation period and the post-period respectively.

Experiment 2.

This experiment was carried out on the black and white goat at a time when she was in fairly full milk, yielding about 600 cc. per day. The experiment was divided into the following periods: (1) 5 days in inside pen; (2) 12 days pre-period; (3) 21 days irradiation, 4 hours per day; (4) 21 days post-period. Collections and analyses were carried out every third day.

Diet.

Food	Weight in g.	% CaO	% P ₂ O ₅
Hay	900	0.9365	0.4560
Oatmeal	300	0.0785	1.0283
Linseed meal	50	0.3889	0.8901
100 g. oatmeal			
Total g. per day	—	7.4103	7.9193

During the irradiation period the goat's appetite increased and the milk yield showed signs of falling off. It was therefore necessary to increase the

¹ Throughout our irradiation experiments we have used these 10 ampere carbon arc lamps. In a previous paper [Henderson, 1925] the current consumption of the arc is wrongly stated [see Mayerson, Gunther and Laurens, 1926].

diet by 100 g. oatmeal per day after the second collection of the light period. Whilst such alteration of the diet is not entirely satisfactory any superimposed influence is probably small. A consideration of the excretion/intake ratio shows a definitely increased absorption of CaO and P_2O_5 during the irradiation period. Thus for CaO the ratios are: pre-period 1.04; light period 0.97; post-period 1.04. The corresponding ratios for P_2O_5 are 1.12, 1.00, 1.04. The decrease in faecal excretion of CaO during irradiation is seen from the following: pre-period 5.56 g. per day; irradiation period 5.39 g. per day; post-period 6.04 g. per day (see also Table I).

The composition of the urine showed little variation throughout the experiment. During the latter half of the irradiation period the milk yield increased, but this was probably due, at least partially, to the increased protein of the diet.

Experiment 3.

The same animal was used as in Exp. 2. In the present experiment, however, she was over 2 months pregnant when the experiment began, and was yielding only about 150 cc. milk per day. The periods of the experiment were as follows: (1) 12 days in inside pen; (2) 16 days pre-period; (3) 16 days irradiation period; (4) 24 days post-period.

Diet. This was identical with Exp. 2 except as regards the composition of the hay which was in the present instance slightly higher in calcium, *i.e.* 1.019 % CaO, 0.370 % P_2O_5 . The total CaO fed per day was 9.69 g. and the P_2O_5 8.36 g.

Results. The balances are summarised in the graph and in Table II. As will be seen from the faecal excretions of CaO and P_2O_5 during the three periods (see Tables I and II) there was again increased absorption during irradiation. Thus the faecal excretions of CaO in the pre-, the irradiation and the post-periods were respectively 8.68, 8.11 and 8.21 g. per day. The corresponding figures for P_2O_5 were 6.75, 5.80, 5.59. The total excretion/intake ratios show a similar trend. Thus for the three periods the ratios are: CaO 1.04, 1.00, 0.99; P_2O_5 0.91, 0.82, 0.81. It is noteworthy that these figures obtained despite the fact that the milk yield continued to rise during the experiment until it was finally about 500 cc. per day.

It will be noted that in this experiment the increased absorption of the irradiation period is continued into the post-period. This is probably due to the fact that during the post-period the goat was in the last quarter of pregnancy.

Experiment 4.

The calcium and phosphorus balances were again followed out in the animal employed in the last experiment, a month after the birth of the kid and at a time when the goat was giving a good milk yield of about 1000 cc. per day.

The periods of the experiment were as follows: (1) 10 days in inside pen; (2) 16 days pre-period; (3) 16 days irradiation period; (4) 16 days post-period. Collections and analyses were made every fourth day.

Diet.

Food	Weight in g.	% CaO	% P ₂ O ₅
Hay	900	1.388	0.476
Oatmeal	100	0.089	1.097
Maize meal	300	0.017	0.758
Linseed meal	50	0.389	0.890
Total g. per day	1350	12.83	8.10

Results. Balance figures are shown in Table II and are represented graphically in Fig. 1. The following figures show that there was a decreased faecal excretion of CaO during the irradiation period: pre-period 8.00, irradiation period 7.85, post-period 8.85 g. per day. The corresponding figures for P₂O₅ excretion are 3.90, 4.20, 4.97 g. per day. The increased absorption, therefore, of calcium which took place during the irradiation period was followed in the post-irradiation period by an increased excretion. The excretion of P₂O₅, however, increased slightly during the irradiation period and continued to increase more rapidly when the irradiation ceased. The significance of these results will be discussed later.

Of the four experiments, in this one alone was any marked increase noted in the urinary excretion of CaO during the light period. Thus the urinary excretion of CaO in g. per 4 days was as follows: pre-period, 0.45, 0.67, 0.56, 0.58; irradiation period, 0.72, 0.91, 0.84, 0.85; post-period, 0.84, 0.67, 0.59, 0.65. We have previously drawn attention to a similar phenomenon in the irradiation of pigs [Henderson, 1925]. Throughout the present experiment the urinary excretion of phosphorus remained fairly constant at the low level characteristic of the ruminant.

DISCUSSION OF RESULTS.

Viewing the results of the four experiments as a whole it will be seen that in all four cases the retention of calcium increases during the irradiation period and falls again during the post-period. The total retention is, of course, influenced by the volume of milk secreted during each period, and in many cases this volume varied considerably. We do not propose to deal with the variations in volume and the small variations in composition in this paper. The effect of such variations upon the total balance is eliminated if the excretion in the faeces is alone considered. Table I shows the percentage of the intake which was excreted in the faeces in the various periods of the four experiments.

From Table II it will be seen that in all four experiments the faecal excretion of calcium was lower in the irradiation periods than in either the pre- or post-periods.

Fig. 1.

Graphs showing amounts of CaO and P₂O₅ in grams retained during the pre-, the irradiation and the post-periods of four experiments.

Table I. % of CaO and P₂O₅ intake excreted in the faeces during each collection period.

Exp. 1		Exp. 2		Exp. 3		Exp. 4	
CaO	P ₂ O ₅	CaO	P ₂ O ₅	CaO	P ₂ O ₅	CaO	P ₂ O ₅
101.8	95.3	81.3	74.2	105.6	92.4	66.5	55.2
97.9	87.5	84.6	93.8	78.0	70.8	62.9	46.1
101.3*	98.9	75.2	78.1	93.4	79.3	58.7	45.9
99.6	122.3	76.9	76.0	101.1	57.2	61.3	47.3
97.3	94.6	74.3	63.6	96.1	82.8	60.0	47.3
99.2	120.4	59.1	57.2	86.1	61.6	62.1	58.2
96.3	94.1	75.3	61.8	83.2	65.4	64.7	54.2
99.1	100.1	76.6	67.9	90.5	75.3	58.2	48.4
100.8	98.1	84.4	97.2	91.1	83.5	51.1	53.5
		70.1	70.5	79.8	67.4	57.5	67.2
		70.5	57.7	80.8	59.3	68.1	61.0
		80.5	74.4	89.3	65.2	99.1	66.8
		78.1	65.4	91.8	63.1		
		90.8	83.3	85.9	66.9		
		87.8	66.9				
		80.6	74.0				
		79.9	78.2				
		73.9	73.3				

* Heavy type indicates end of a period.

Table II. Average daily intake, output and balance in g.

	CaO					
	Intake	Output				Balance
		Milk	Urine	Faeces	Total	
<i>Exp. 1</i>						
Pre-period (11 days)	11.73	0.40	0.13	11.77	12.30	-0.57
Irradiation period (14 days)	11.73	0.35	0.13	11.53	12.01	-0.28
Post-period (5 days)	11.73	0.36	0.11	11.69	12.16	-0.43
<i>Exp. 2</i>						
Pre-period (12 days)	7.00	1.46	0.29	5.56	7.31	-0.31
Irradiation period (21 days)	7.38	1.50	0.29	5.39	7.18	+0.20
Post-period (21 days)	7.39	1.43	0.22	6.04	7.69	-0.30
<i>Exp. 3</i>						
Pre-period (16 days)	9.16	0.42	0.41	8.68	9.51	-0.35
Irradiation period (16 days)	9.13	0.62	0.42	8.11	9.15	-0.02
Post-period (24 days)	9.49	0.78	0.36	8.21	9.35	+0.14
<i>Exp. 4</i>						
Pre-period (16 days)	12.82	2.80	0.15	8.00	10.95	+1.87
Irradiation period (16 days)	12.82	2.35	0.21	7.85	10.41	+2.41
Post-period (16 days)	12.82	2.10	0.17	8.85	11.12	+1.70
	P ₂ O ₅					
	Intake	Output				Balance
		Milk	Urine	Faeces	Total	
<i>Exp. 1</i>						
Pre-period (11 days)	5.10	0.55	0.05	4.80	5.40	-0.30
Irradiation period (14 days)	5.10	0.48	0.05	5.63	6.16	-1.06
Post-period (5 days)	5.10	0.52	0.04	5.10	5.66	-0.56
<i>Exp. 2</i>						
Pre-period (12 days)	6.72	2.08	0.06	5.41	7.55	-0.83
Irradiation period (21 days)	7.62	2.34	0.05	5.20	7.59	+0.03
Post-period (21 days)	7.91	2.34	0.07	5.83	8.24	-0.33
<i>Exp. 3</i>						
Pre-period (16 days)	8.18	0.69	0.04	6.75	7.45	+0.73
Irradiation period (16 days)	8.16	0.85	0.04	5.80	6.69	+1.47
Post-period (24 days)	8.26	1.06	0.05	5.59	6.70	+1.56
<i>Exp. 4</i>						
Pre-period (16 days)	8.09	3.61	0.03	3.90	7.54	+0.55
Irradiation period (16 days)	8.09	3.12	0.03	4.20	7.35	+0.74
Post-period (16 days)	8.09	2.60	0.03	4.97	7.60	+0.39

This relationship does not hold as regards the faecal phosphorus. In Exp. 1 its excretion is higher in the irradiation period than in either the pre- or post-period. In Exp. 2 the excretion is least in the irradiation period. In Exp. 3 the excretion of phosphorus in the faeces decreases progressively from the pre-period through the irradiation period to the post-period. Coupling this with the fact that in the post-period the faecal excretion of calcium is very little more than in the irradiation period we are inclined to attribute the tendency to a rising retention in Exp. 3 to the fact that the goat was pregnant. It would appear that irradiation gave an impetus to the natural process of mineral storage characteristic of pregnancy. One of us [Magee, 1924] has shown that just about this time (*i.e.* 14th week) the basal metabolic rate begins to rise rapidly in the pregnant goat. Hoffström [1910] found in experiments on pregnant women that the beginning of rapid growth of the fetus (in the 29th week) was accompanied by decreased excretion of calcium in the faeces of the mother. In Exp. 4 the faecal phosphorus gradually increased, this increase being most marked in the post-period. Considering this in conjunction with the marked increase in faecal excretion (1 g. per day) of CaO in the post-period it would appear that the goat was tending rapidly towards a negative balance, and that this tendency had been temporarily checked by irradiation. Despite the fact that the goat was in very good condition the initial positive balances of CaO and P_2O_5 in this experiment are unusual in view of the stage of lactation. We are unable to say to what extent they are contributed to by (1) the fact that following upon the post-period of Exp. 3 the goat was irradiated for 16 days (4 hours per day) in an investigation of the effect upon the composition of the milk, and (2) the quality of the hay. To our knowledge there had been no difference in the method of curing, but the calcium, phosphorus and nitrogen contents were all high.

As regards the calcium and phosphorus balances there is only a rough parallelism. Throughout all four experiments the phosphorus balances are less consistent. We have repeatedly noted in metabolism experiments on pigs that whilst the graphs of phosphorus and calcium retention may run parallel for a time the phosphorus curve occasionally, for no obvious reason, shows an unexpected transitory rise or dip. Forbes *et al.* [1922] in their metabolic experiments on cows draw attention to the "partial independence" of calcium and phosphorus.

In our work on pigs we [Orr, Magee and Henderson, 1924] noted that decreased faecal excretion of calcium or phosphorus in the irradiation period may be accompanied by an increased urinary excretion. Orr, Holt, Wilkins and Boone [1923] had found a similar effect in the irradiation of rachitic children; Mayerson, Gunther and Laurens [1925] have observed an increased urinary excretion of calcium and phosphorus on irradiating normal dogs. The figures of Hart, Steenbock and Elvehjem [1924] suggest a like relationship between faecal and urinary excretion in irradiated goats. In our present experiments the only point at which this effect is clearly brought out is the

calcium excretion of Exp. 4. Here the decreased faecal excretion of calcium in the irradiation period is accompanied by a corresponding rise in the urinary excretion of calcium. It is noteworthy that of the four experiments number 4 is the only one in which the calcium balance is positive throughout the experiment.

The results of Hart, Steenbock and Elvehjem [1924] and of the present experiments which indicate that the calcium, and to some extent, phosphorus retention during lactation may be definitely increased by ultra-violet irradiation suggest important practical applications. Whilst the negative balances of the first half of lactation may be largely physiological they are no doubt partially an adaptation to the artificial conditions of domestic milking animals. They probably, therefore, border closely upon the pathological, particularly in heavy milkers. Forbes and his associates [1922] have, for instance, pointed out that forced milk production in cows results in a loss of breeding capacity. Hart and his colleagues [1921, 1922, 1, 2] have observed "disaster in reproduction with cows where probably negative calcium balances are long continued." Orr [1923], moreover, has suggested that there is probably a relationship between high milk yield and susceptibility to tuberculosis. Lastly, Fingerling [1911] has shown that reduction of calcium and phosphorus in the diet of milking goats eventually causes the milk yield to fall. The last two points receive some support from a recent experiment carried out at this Institute by Orr, Crichton and Middleton [1925]. A group of heavy milking cows had calcium carbonate, sodium chloride, iron oxide and potassium iodide added to their rations. The amounts of the salts fed were determined by the milk yield of the cows. Another group of cows on the basal ration alone was used as controls. During the first lactation little difference was observed between the groups. In the second lactation the "mineral" group increased in milk yield whilst the yield of the "non-mineral" group diminished. Further, whilst several of the "non-mineral" cows reacted positive to the tuberculin test those of the "mineral" group were all negative.

In general, therefore, there seem some grounds for concluding that depletion of minerals in lactating animals may adversely affect breeding and milk production and may increase susceptibility to tuberculosis. Further, whilst in minimising such depletion the composition of the diet is of prime importance, ultra-violet irradiation would seem to be a valuable adjuvant.

SUMMARY.

(1) In four balance experiments on lactating goats, irradiation by the carbon arc lamp reduced the faecal excretion of calcium. The effect upon the excretion of phosphorus was less constant. It seems probable that irradiation increases the stimulus to calcium storage during pregnancy and diminishes the calcium loss during full lactation.

(2) A definite increase in the urinary excretion of calcium on irradiation was only noted in Exp. 4 in which there was already retention of calcium.

(3) The influence of ultra-violet light in minimising the depletion of minerals is briefly considered with respect to certain pathological conditions which may occur in heavy milking animals.

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